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Phytochemicals and antioxidant activities of *Rhus tripartitum* (Ucria) fruits depending on locality and different stages of maturity

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ABSTRACT

The phytochemical content (total phenolic compounds, total flavonoids, condensed tannins and phenolic composition) and the antioxidant potential of *Rhus tripartitum* fruits collected from different localities were screened during maturity. Significant variability was detected. HPLC analyses revealed the presence of 24 compounds with notable differences. Flavone and betulinic acid, which have numerous benefits, were the main detected compounds (more than 73%). This work highlights the importance of *R. tripartitum* fruits as dietary sources of natural antioxidants, and might be appropriate for the development of reliable index to estimate fruit richness with bioactive molecules.

1. Introduction

Rhus species, which often grow in non-agriculturally viable regions, are widely used in food, and in modern and traditional medicine (Lee et al., 2010; Wu et al., 2013). The antimalarial, antiviral, antimicrobial, antitumorigenic, and atherosclerosis properties for these species have been reported (Ahmed et al., 2001; Choi et al., 2012; Lee et al., 2010; McCutcheon, Ellis, Hancock, & Towers, 1992; Zargham & Zargham, 2008). *Rhus tripartitum* is a local pre-saharan Tunisian plant that grows largely under rainfall ranging between 100 and 600 mm/year and at altitudes ranging from 10 to 500 m (Pottier-Alapetite, 1979). The fruits of this species can be consumed fresh or stored. They are mixed to drinking water for an acceptable taste (Le Floc'h & Boulous, 1983).

Phytochemicals, especially phenolic compounds as secondary metabolites, are of great importance due to their beneficial effects as anticarcinogenic, antithrombotic and anti-inflammatory (Chung, Wong, Huang, & Lin, 1998). Currently, food scientists and nutrition specialists suggest that phytochemicals offer many health benefits when consumed as part of the usual diet (Halliwell & Gutteridge, 1989). Moreover, due to the possible negative effect of synthetic antioxidants, food industries pay much attention to natural antioxidants, which can be used as food additives or as pharmaceutical supplements (Nicoli, Anese, & Parpinel, 1999). In addition, it has been reported that person who eat daily more than five fruits and vegetables have approximately half the risk of developing a large variety of cancer forms (Gescher, Pastorino, Plummer, & Manson, 1998). While, the content of phytochemicals and the antioxidant capacity of fruits are influenced by numerous factors such as sunlight, soils, season, region of cultivation, fruit variety, and stages of maturity (Chirinos, Pedreschi, Rogez, Larondelle, & Campos, 2013; Ilahy, Hdider, Lenucci, Tlili, & Dalessandro, 2011; Villa-Rodríguez, Molina-Corral, Ayala-Zavala, Olivas, & González-Aguilar, 2011). The maturity stage is an important factor that influences the compositional quality and the quantity of fruits. Due to the fact that during maturation, several variations (biochemical, physiological and structural) take place and determine the fruit's quality (Siddiqui et al., 2013).

To our knowledge there are no reports about influence of different geographical region and maturity stages on phytochemical content and antioxidant activity of *R. tripartitum* fruits. So, the goal of this work was to study the differences in amount and composition of phenolic compounds and to estimate the antioxidant activities of *R. tripartitum* with respect to geographical region and maturity stages.

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2. Material and methods

2.1. Plant material

Fruits of *R. tripartitum* were harvested from two Tunisian regions, Dkhila (36° 52'; 9° 42') and Ain Jalloula (35° 47'; 9° 47'), during February and March 2013. Fruits were picked up according to external color; yellow were chosen as immature stage, mahogany brown as the intermediate stage and dark brown as the last stage of maturity (mature stage). Samples were air-dried at room temperature and then ground for further analysis. Vouchers specimens are deposited in the herbarium of National Institute for Research in Rural Engineering Water and Forests (INRGREF).

2.2. Methanolic extracts

The powder was extracted and stirred with MeOH at 30 °C for one night. Then a Whatman No. 1 filter paper was used to remove the particles. The residue was more time extracted, filtered and concentrated.

2.2.1. Total phenolic content

Total phenols were determined using the Folin–Ciocalteu method described by Tlili et al. (2013). To 0.125 mL of diluted methanolic extract, 0.5 mL of Folin–Ciocalteu reagent and 1.25 mL of Na_2CO_3 (7%) were added. The tubes were incubated for 90 min in the dark, and then the absorbance was measured at 760 nm. The analyses were performed in triplicate. Total phenolic, expressed as mg gallic acid equivalents per g dry residue (mg GAE/g DR), were calculated using gallic acid standard curve (concentration range: 50–200 μg/mL).

2.2.2. Total flavonoid content

Total flavonoid content was estimated using the method reported by Dewanto, Wu, Adom, and Liu (2002). 250 μL of the methanolic extract was mixed with 75 μL of $NaNO_2$ (7%) and then 150 μL of $AlCl_3$ (10%) was added. After 5 min, 0.5 mL of NaOH (1 M) was added to the mixture. The absorbance was measured at 510 nm after incubation at room temperature for 15 min. The analyses were performed in triplicate. Total flavonoids, expressed in mg of quercetin equivalent per gram dry residue (mg QE/g DR), were calculated with respect to quercetin standard curve (concentration range: 100–750 μg/mL).

2.2.3. Condensed tannins contents

To determine the procyanidins content the method described by Sun, Richardo-da-Silvia, and Spranger (1998) was used. 50 μL of the methanolic extract was mixed with 3 mL of methanol vanillin solution (4%) and 1.5 mL of concentrated H_2SO_4. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm. The analyses were performed in triplicate. The quantity of proanthocyanidins, expressed as mg catechin equivalent per gram dry residue (mg CE/g DR), was determined using a catechin calibration curve (100–750 μg/mL).

2.2.4. Determination of total antioxidant capacity (TAC)

The total antioxidant capacities of the methanolic extract were estimated using the assay described by Prieto, Pineda, and Aguilar (1999). 0.1 mL of extract was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After incubation in boiling water bath for 90 min, the absorbance was measured at 695 nm. Quercetin was used as reference compounds. The total antioxidant capacity was expressed as the mg of gallic acid equivalents per gram of dry residue (mg GAE/g DR).

2.2.5. Reducing power assay (RPA)

The reducing power of the extracts was determined according to the method reported by Oyaizu (1986). To 2.5 mL of methanolic extract we added 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $K_3Fe(CN)_6$ (freshly prepared). After incubation at 50° for 20 min the mixture was centrifuged at 650*g* for 10 min and then 2.5 mL of trichloroacetic (10%) were added. 2.5 mL of the supernatant was mixed with 2.5 mL distilled water and 0.5 mL of $FeCl_3$ (0.1%). The absorbance was measured at 700 nm. The EC_{50} value (μg/mL) was the effective concentration at which the absorbance was 0.5% for the reducing capacity. Vitamin C was used as reference standards.

2.2.6. Scavenging ability towards DPPH radical (DPPH. assay)

The scavenging activity on DPPH radical of methanolic extracts was estimated as reported by Tlili et al. (2013). 0.5 mL of different concentrations of the methanolic extract was mixed with 3.8 mL of methanolic solution of DPPH (6 μM). After shaking vigorously, the mixture was left to stand for 30 min in the dark at room temperature. The absorbance was measured at 517 nm. The percentage inhibition (*I%*) of free radical DPPH was calculated with the following formula:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] * 1000$$

DPPH scavenging activity is presented by IC_{50} value, defined as the concentration of the sample required to scavenge 50% of free radicals present in the test solution. BHT was used as reference compounds.

2.2.7. $ABTS^+$ free radical scavenging activity

$ABTS^+$ assay was determined according to the method described by Tlili et al. (2013). $ABTS^+$ radical cation was produced by mixing 7 mM ABTS solution with 2.45 mM potassium persulphate and the mixture was allowed to stand in the dark at room temperature. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then 25 μL of the sample or trolox standard was added to 2 mL of diluted $ABTS^+$ solution. The absorbance at 734 nm was measured. Trolox was used as reference standards.

2.2.8. High performance liquid chromatography analysis

To analyze the different compound in the methanolic extract using HPLC an HP 1100 series coupled with an UV–Vis multiwavelength detector was used. A reversed phase column Hypersil ODS-C18 (250 × 4.6 mm i.d., 4 μm, Algilent, USA) was connected with a Hypersil ODS-C18 guard column (125 × 4.6 mm i.d., 4 μm, Algilent, USA). Sample and mobile phase were filtrated through a 0.45 μm Millipore filter type GV (Millipore, Bedford, MA) prior to HPLC injection. To elute the samples at ambient temperature, a gradient of acetonitrile–water (solvent A) and 0.2% sulphuric acid–water (solvent B) were used. The elution gradient program with a ratio of A to B was as follows: from 0 to 12 min (15:85, v/v), from 12 to 14 min (40:60, v/v), from 14 to 18 min (60:40, v/v), from 18 to 20 min (80:20, v/v), from 20 to 24 min (90:10, v/v) and from 24 to 28 min (100:0, v/v). The flow rate was kept at 0.5 ml/min and 10 μl of sample was injected. The different detected compounds were identified by comparing their retention time with those of injected authentic standards. Analyses were performed in duplicate.

2.3. Statistical analyses

Each value was the mean ± SD of three experiments. The Pearson's correlation between these mean values was performed by SPSS statistical package (version 17.0). The test was considered significant when p value is lower than 0.05.